

II. Mantoux Tuberculin Skin Testing and Interferon Gamma Release Assays (IGRAS)

The Mantoux tuberculin skin test (TST) and interferon gamma release assays (IGRAs) are tests for the presence of infection with *Mycobacterium tuberculosis*. These tests should be performed in persons who are at risk of infection with *M. tuberculosis* and/or at high risk for progression to active TB if infected. These tests have a number of limitations:

- They do not distinguish between latent tuberculosis infection (LTBI) and active TB disease
- Both false-negative (negative results in persons truly infected with *M. tuberculosis*) and false-positive (positive results in persons not infected with *M. tuberculosis*) results may occur
- They have issues with test-retest reliability, so if the same person is tested on more than one occasion, a different result may be obtained despite no change in TB infection. These issues arise from multiple sources, including reader variability (TST), biologic variability, specimen handling (IGRAs), and laboratory variability (IGRAs)
- Testing of low-risk populations is likely to yield many false-positive results, resulting in unnecessary treatment

At the time of this writing, two IGRAs are commercially available in North Carolina: the Quantiferon Plus® and T-SPOT.TB®. For most purposes these tests are roughly equivalent, and either can be used when an IGRA is indicated.

While either a TST or an IGRA may be used to screen for latent TB, in certain situations one test may be preferred. The decision to perform a TST or an IGRA will depend on a number of factors, including cost, logistics, and local test availability. The table below provides general guidance as to which type of test would be preferred in different clinical situations.

Situation	Preferred test
Person 2 years or older born outside the U.S.	IGRA
Person known to have received BCG (vaccine or bladder cancer treatment)	IGRA
Person unlikely to return for TST reading	IGRA
Child under 2 years old	TST*
Person at low risk for TB infection (e.g. administrative screening)	IGRA
Testing in settings where the TST is infrequently performed	IGRA

* Emerging evidence suggests that IGRAs may reduce false-positive tests among BCG-vaccinated children, so an IGRA is a reasonable alternative for such children

Mantoux skin testing

The Mantoux tuberculin skin test is the only skin test recommended for latent TB screening by NC TB Control.

- The TST should be repeated when interpretations are ambiguous or inconclusive.
- Under nursing regulations, an RN or LPN may not delegate the administration or reading of a TST to an unlicensed person.
- Healthcare workers responsible for placing and reading TSTs should receive classroom training about TB transmission, pathogenesis, testing and treatment of active tuberculosis and latent TB infection. Additionally, the healthcare worker should be observed placing and reading at least 10 positive TST's and 10 negative TST's. A copy of the Quality Control (QC) procedural observation checklists (Appendix F) can be found at the end of this chapter

A. Administration

1. Use 0.1 cc of 5TU Purified Protein Derivative (PPD)
2. Use tuberculin syringe with a 3/8-inch, 26-27 gauge needle
3. Clean volar or flexor surface of left forearm approximately 2-4 inches below the elbow; allow to dry completely
4. Give intradermal injection with needle bevel upward; a tense, white wheal of 6-10mm in diameter should be produced when the TST is accurately administered.
5. Repeat injection at another site at least 2 inches away if part of the antigen is lost or the injection is given too deeply. Specify location of the retest in the record.
6. Follow your agency blood borne pathogens guidelines for standard precautions
7. Tuberculin solution can be adversely affected by exposure to light or temperature extremes. For storage and handling, **see Chapter VI., C. 1.**
8. Administer TST prior to or simultaneously with live virus vaccines, e.g., measles, mumps, rubella, smallpox and chicken pox. If the TST is not given simultaneously, wait 4-6 weeks after these vaccines before giving the TST.
9. Immediate hypersensitivity reactions to tuberculin (redness, swelling, pruritus, heat) can occur shortly after injection and usually disappear within 24 hours. This has no clinical significance and is not considered a positive test.

10. There is no contraindication to repeating a TST that was previously positive; a TST should be administered if there is no documentation of a prior mm reading.
11. TST is both safe and reliable throughout the course of pregnancy
12. TST is safe for infants of any age. A negative reading is considered valid for infants at least 6 months of age or older (adjust age for premature infants). A positive reading is valid at any age.
13. TST is not contraindicated for individuals who have been vaccinated with BCG, but as noted above IGRAs are preferred for most such individuals

B. Reading

1. Read TST 48-72 hours after placement, preferably at 72 hours
 - a. Instruct individuals to return to the health department if induration occurs after the TST is read
 - b. positive TST reactions occurring after 72 hours are considered valid
 - c. negative TST reactions should be repeated when individuals fail to return within 72 hours
2. Locate induration (not redness) by palpating in a crosswise motion
3. Measure transversely (crosswise or “east to west”) to the long axis of the forearm and record this as a single measurement
4. Record reaction in mm (example: 0mm, 16mm) and document date of reading and signature of person reading the test
5. Cold packs or over the counter topical steroid preparations (e.g. 1% hydrocortisone) may be used for the relief of pruritus and local discomfort
6. Evidence of severe scarring at an old TST site denotes a prior positive reaction and a repeat TST may not be indicated

C. Interpretation

1. A reaction of ≥ 5 mm induration is considered positive for:
 - a. close contacts to an individual with known or suspected infectious tuberculosis within the past 2 years
 - b. those suspected of having active TB disease based on clinical and/or chest x-ray evidence
 - c. individuals with HIV infection

- d. individuals with fibrotic changes on chest x-ray consistent with prior TB
 - e. individuals with organ transplants and other immunosuppressed patients, including those receiving \geq 15 mg per day of Prednisone for one month or longer or persons taking or considering taking tumor necrosis factor (TNF) inhibitors such as etanercept (Enbrel®), infliximab (Remicade®), adalimumab (Humira®) or anakinra (Kineret™), or other similar biologic immunosuppressive agents
2. A reaction of \geq 10mm induration is considered positive for:
- a. children younger than 4 years of age
 - b. foreign-born individuals from high-prevalence countries, e.g. Asia, Africa, Caribbean, Latin America, Mexico, South America, Pacific Islands or Eastern Europe
Low-prevalence countries for TB disease are USA, Canada, Japan, Australia, Western Europe and New Zealand
 - c. HIV-negative individuals who inject illicit drugs or use crack cocaine
 - d. individuals with medical conditions that have been reported to increase the risk of tuberculosis disease once infected:
 - diabetes mellitus
 - chronic malabsorption syndrome
 - chronic renal failure
 - leukemia, lymphomas, Hodgkin's disease
 - cancer of the head or neck
 - weight loss of \geq 10% below ideal body weight
 - silicosis
 - gastrectomy, or jejunioileal bypass
 - e. residents and staff in long-term care facilities
 - f. health care workers
 - g. inmates in the Department of Corrections
 - h. staff with direct inmate contact in the Department of Corrections and jails
 - i. employees of HIV/AIDS adult daycare centers
 - j. homeless shelter residents, employees and volunteers
 - k. individuals who increase their mm reading by 10mm or more within 2 years (converter)
 - l. mycobacteriology lab personnel
 - m. children and adolescents exposed to high-risk adults (homeless, substance abuse, incarcerated, HIV positive)
 - n. persons who have traveled outside the US and stayed with family and friends who live in high incidence areas, for greater than 1 month cumulatively
3. A reaction of \geq 15mm induration is considered positive for:

- a. individuals who do not have any of the above risk factors

D. False Negative TST Reactions

An individual may be infected with *M. tuberculosis* but have little or no reaction to TST. False negative reactions may occur in any patient, but are more common in the following situations:

1. Recent viral infections (rubella, mumps, influenza, measles, chicken pox)
2. Overwhelming tuberculosis disease

Note: This means that a negative TST in the work-up of a possible case of TB does NOT rule out TB.

3. Immunosuppression associated with old age, debility, malnutrition, HIV infection, medications, and malignancy.

Note: Routine anergy testing is not recommended for use in identifying TB infection in immunosuppressed individuals due to the antigen tests' questionable validity and unpredictable variability over time.

4. Very recent tuberculosis infection (the individual may not have had time to develop the delayed hypersensitivity reaction, which can take up to 8 weeks after exposure)
5. Recent (within 4-6 weeks) immunization with certain live virus vaccines (measles, mumps, rubella, chicken pox, smallpox)
6. High-dose steroids (≥ 15 mg of prednisone or its equivalent given daily for one month or longer) and other immunosuppressive agents
7. Infants younger than 6 months old (may have false-negative reactions because their immune systems are not fully developed)
8. Improper antigen storage, handling, technique in administration or error in reading

E. False Positive TST Reactions

An individual may not be infected with *M. tuberculosis*, but have a false positive reaction. False positive reactions may be caused by:

1. Cross reactions resulting from infection with nontuberculous mycobacteria
2. BCG (bacille Calmette-Guérin) vaccine
3. Reading erythema (redness) rather than induration

F. TST Converters

An increase in reaction size of ≥ 10 mm within a period of 2 years should be considered a converter

G. Two-Step TST (Booster Phenomenon)

- in some individuals, the ability to react to the TST may gradually diminish over time. If skin tested at this point, these individuals may have a false negative reaction. However, if retested within 1 week to 1 year they may then demonstrate a positive reaction (the "booster" phenomenon). The booster phenomenon may occur at any age, but is more common in older persons.
- two-step testing reduces the likelihood of interpreting a "boosted" reaction as a true conversion or a new infection; it is recommended in situations where there will be repeat testing on a regular basis. Two step testing is required for staff and residents of long term care facilities as well as staff in adult day care centers that provide care for HIV/AIDS clients (see Chapter XI -10A NCAC 41A .0205 (c)1- 4. If the individual has had a documented TST within the last 12 months, that TST can be counted as the first step in 2 step testing.
- if the reaction to the first test is positive, consider the individual infected
- if the reaction to the first test is negative a second test should be given 1 to 3 weeks later:
 1. if the second test is positive, consider the individual infected
 2. if the second test is negative, consider the individual not infected
- record reactions in mm and document dates of reading and signature(s) of person(s) reading the tests
- Two step is not needed when using IGRA's instead of TST's

H. Bacille Calmette Guerin (BCG)

BCG is an attenuated or weakened form of tuberculosis, originally derived from *Mycobacterium bovis*, and is used in high-incidence countries to protect infants and young children against severe forms of TB disease (miliary and meningeal disease). BCG is also used as an intravesicular therapy (instilled into the bladder) for treatment of bladder cancer. BCG (identified as *M. bovis*) can frequently be isolated from the urine of persons who have recently received intravesicular therapy, but this does not always require treatment.

Both BCG vaccination and intravesical BCG can cause a reactive TST; this effect seems to wane over time but can persist for years. IGRAs are

therefore preferred for most persons who have received the BCG vaccine.

I. Candidates for TST

- TST of individuals and groups should be undertaken only if the diagnostic evaluation and a course of preventive therapy can be completed
 - **routine testing of low-risk individuals is not recommended; locally purchased PPD must be used for all low-risk testing, e.g., job-related**
 - ***state supplied PPD may be used only for persons in categories #1-7 and #9 as follows (high-risk for infection or disease)***
1. The following children and adults are legally required (10A NCAC 41A.0205) to receive a TST:
 - a. household and other close contacts of active cases of pulmonary and laryngeal tuberculosis
 - b. persons reasonably suspected of having tuberculosis disease
 - c. inmates in the custody of, and staff with direct inmate contact, in the Department of Corrections upon incarceration or employment, and annually thereafter
 - d. patients and staff in long term care facilities upon admission or employment, using the two-step skin test method
 - e. staff in adult day care centers providing care for persons with HIV infection or AIDS upon employment, using the two-step skin test method
 - f. persons with HIV infection or AIDS
 2. The following children and adults should receive a baseline TST when they initially present for health care:
 - a. foreign-born individuals from high incidence areas, such as, Asia, Africa, Caribbean, Latin America, Mexico, South America, Pacific Islands or Eastern Europe
Low-prevalence countries for TB disease are USA, Canada, Japan, Australia, Western Europe and New Zealand
 - b. individuals who inject illicit drugs or use crack cocaine
 - c. migrants, seasonal farm workers, and the homeless (if unable to ensure completion of evaluation and TLTBI, screen for disease)
 - d. persons who have traveled outside the US and stayed with family and friends who live in high incidence areas, for greater than 1 month cumulatively

- e. children and adolescents exposed to high-risk adults (homeless, substance abuse, incarcerated, HIV positive)
- f. persons with conditions that increase the risk of progression to disease once infected
 - diabetes mellitus
 - chronic renal failure
 - chronic malabsorption syndrome
 - leukemia, lymphomas, Hodgkin's disease
 - cancer of the head or neck
 - silicosis
 - weight loss of > 10% ideal body weight
 - gastrectomy or intestinal bypass
 - current or planned use of immunosuppressive medications, particularly biologic agents (e.g. infliximab, adalimumab, etanercept)

A subsequent TST is not necessary unless there is a continuing risk of exposure to persons with tuberculosis disease

3. **Persons taking or considering taking tumor necrosis factor (TNF) inhibitors (e.g., etanercept, infliximab, adalimumab or anakinra) which can suppress the immune system are at high risk for TB disease if infected. Before starting these drugs, a TST or IGRA should be done (preferably a 2-step test if the TST is used) and treatment for LTBI started if the TST reading is 5 mm or greater or the IGRA is positive. If there is a credible history of exposure to TB, TLTBI should be initiated regardless of TST result.**
4. Clinically assess all household members in the immediate environment of a child ≤ 2 years of age with a newly identified positive TST or IGRA to rule out an undiagnosed case of tuberculosis. An assessment should include an evaluation of symptoms for TB disease and may include a TST or IGRA, bacteriological examination and chest x-ray, if indicated.
5. Clinically assess all household members in the immediate environment of a pregnant woman with a newly identified positive TST to rule out TB exposure in the immediate environment that the newborn infant will be entering. An assessment should include an evaluation of symptoms for TB disease and may include a TST or IGRA, bacteriological examination and chest x-ray, if indicated.
6. Homeless shelters
 - a. staff and volunteers should be educated regarding the symptoms of tuberculosis disease. Those clients with symptoms suggesting TB disease should be promptly evaluated for active disease.

- b. staff and volunteers should receive a two-step TST or IGRA on employment
 - c. routine TST or IGRA of clients should be undertaken only if the diagnostic evaluation and course of preventive therapy can be initiated and completed
7. Local jails and detox units
- a. All jail facilities must conduct a facility specific risk assessment; for additional guidance see: prevention and Control of Tuberculosis in Correctional and Detention Facilities: Recommendations from CDC. MMWR 2006;55 (No RR-9)
 - b. Test staff with a TST (using the two-step method) or IGRA upon employment. Annual TST or IGRA is based on the facility's current risk level.
 - c. Verbally screen all inmates for symptoms of TB on admission. Inmates with symptoms suggesting TB disease should be evaluated for active disease
8. Staff and residents in occupational settings where TST is required by regulatory and/or agency policy
- a. two-step TST [upon admission or employment] individuals who cannot provide a documented negative TST within the preceding 12 months:
 - individuals who can provide a documented negative TST within the preceding 12 months should receive a single TST and use this result as the second part of the two-step test
 - individuals who can provide a documented positive TST should have a Record of Tuberculosis Screening (DHHS 3405) completed using the most recent chest x-ray report
 - b. individuals with a previously documented positive TST should be re-x-rayed only when symptoms for tuberculosis disease are present
9. Refugee Notification of Arrival: Class A and B Conditions (also see chapter IX -18 for reporting in NCEDSS)
- a. prior to entering this country, federal regulations require newly arrived refugees \geq 15 year of age* to have a medical exam consisting of a chest x-ray. Sputum smears are only done if the chest x-ray indicates possible TB disease. The overseas exam may not include a TST or sputum for culture. Furthermore the required chest x-ray may have been done up to 2 years prior to arrival in the US

Because of these uncertainties it is recommended that each refugee be fully evaluated for TB disease, including a TST and chest x-ray if not done overseas. If a chest x-ray was taken overseas, repeat chest x-ray if the original is of poor quality or x-ray is > 6 months old. Sputum should be obtained for smear and culture, if indicated. When TB disease is ruled out, consider treating for LTBI if indicated

- b. refugees arriving in this country without an A or B classification status (normal chest film) who are determined to have a positive TST or IGRA during their initial evaluation should have a new chest film taken before starting treatment for LTBI
- c. Class A TB with waiver
 - Applicants who have tuberculosis disease and have been granted a waiver
- d. Class B1 TB, Pulmonary, No Treatment
 - Applicants who have medical history, physical exam, or chest x-ray findings suggestive of pulmonary tuberculosis but have negative AFB sputum smears and cultures and are not diagnosed with tuberculosis or can wait to have tuberculosis treatment started after immigration
- e. Class B1 TB, Pulmonary, Completed Treatment
 - Applicants who were diagnosed with pulmonary tuberculosis and successfully completed directly observed therapy prior to immigration. The cover sheet should indicate if the initial sputum smears and cultures were positive and if drug susceptibility testing results are available
- f. Class B1 TB, Extrapulmonary
 - Applicants with evidence of extrapulmonary tuberculosis.
- g. Class B2 TB LTBI Evaluation
 - Applicants who have a tuberculin skin test ≥ 10 mm or positive IGRA but otherwise have a negative evaluation for tuberculosis. The size of the TST reaction or IGRA result, the applicant's status with respect to LTBI treatment, and the medication(s) should be documented on the overseas evaluation form. Contacts with a TST ≥ 5 mm or positive IGRA should receive this classification if they are not already classified as a Class B1 TB, Pulmonary. For
- h. Class B3 TB, Contact Evaluation
 - Applicants who are a recent contact of a known tuberculosis case.

J. Chest x-rays

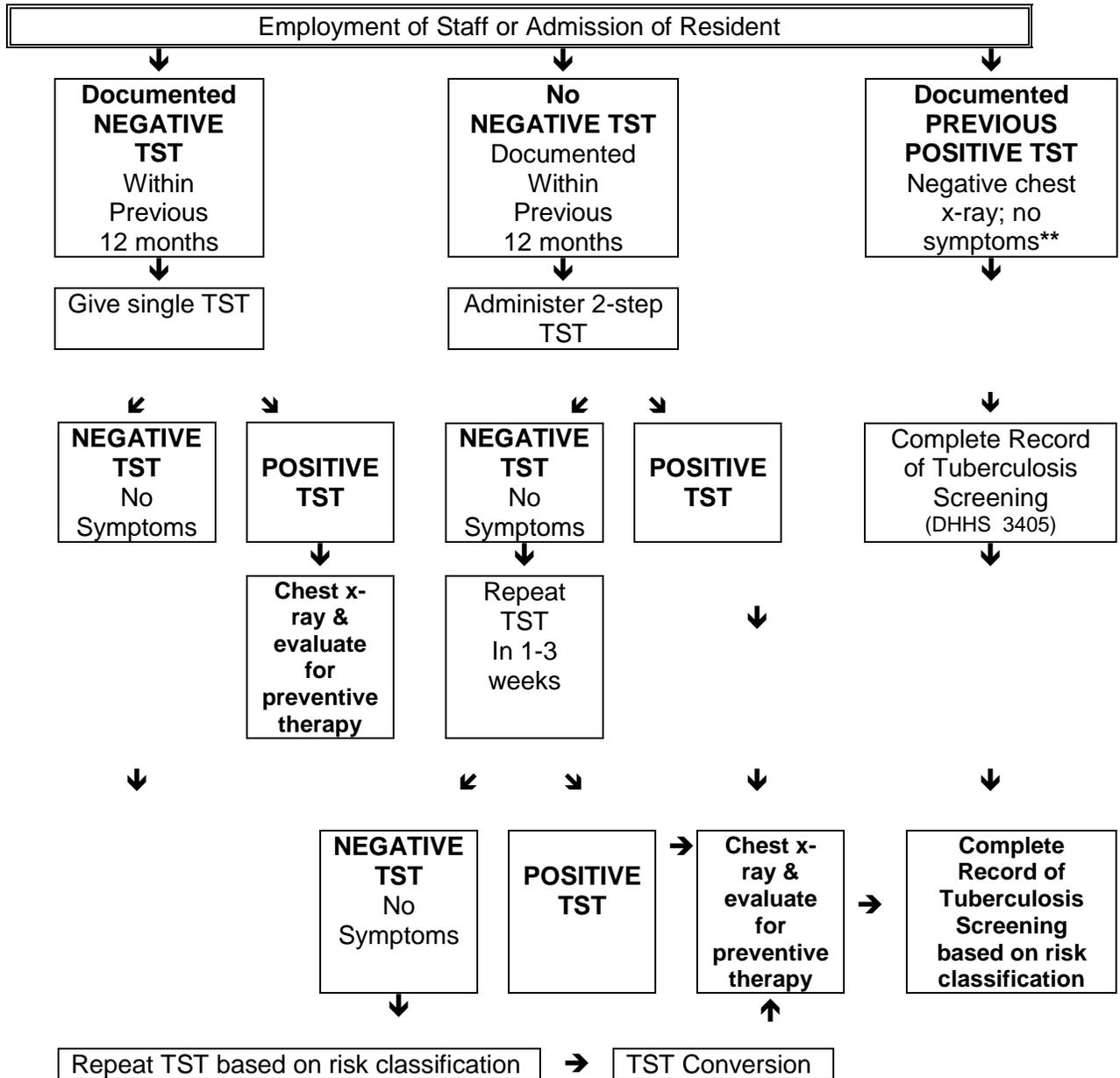
1. A posterior-anterior view of the chest should be obtained on all adults:
 - a. with a newly identified positive TST or IGRA
 - b. with symptoms suggestive of TB disease regardless of TST/IGRA results
 - c. with suspected extrapulmonary TB disease
 - d. with negative TST/IGRA and starting treatment for LTBI, i.e. close contact

2. A posterior-anterior and lateral view of the chest should be obtained on children under 5 years of age:
 - a. with a newly identified positive TST/IGRA
 - b. with symptoms suggestive of TB disease regardless of TST/IGRA results
 - c. with suspected extrapulmonary TB disease
 - d. with negative TST/IGRA and starting latent TB treatment, i.e. close contact

3. For chest x-rays during pregnancy, see Chapter III, page 5

4. Individuals with a previously documented positive TST/IGRA and a negative chest x-ray should have a repeat x-ray only when symptoms for tuberculosis disease are present (see Chapter III for chest x-ray recommendations when starting treatment for LTBI for someone with a remote or prior positive TST/IGRA)

K. Two-Step Tuberculin Skin Testing (TST) *



* Initial two-step TST is required for employees and residents of long-term care facilities and facilities providing adult day care for HIV positive individuals. Other agency/institution requirements may vary.

** Repeat x-ray ONLY if signs and symptoms of tuberculosis disease are present. Obtain sputums and x-ray individuals with symptoms regardless of TST result.

L. Interferon Gamma Release Assays (IGRAs)

Background

Interferon gamma release assays (IGRAs) are relatively new tests for tuberculosis (TB) infection. These tests measure the patient's immune response (interferon gamma release) after stimulation of white blood cells in a test tube with 2-3 relatively TB-specific antigens. In contrast to the tuberculin skin test, which requires two separate visits for placement and reading, IGRAs offer the possibility of testing for TB infection with a single blood draw at a single visit. In addition to the logistical advantage of requiring a single visit, IGRAs may have other advantages over the tuberculin skin test. The antigens used for the IGRA tests are not present in the Bacille Calmette-Guerin vaccine (BCG), so false positive tests due to BCG are unlikely to occur. The IGRA antigens are also not present in most nontuberculous mycobacteria, so false positive tests due to nontuberculous mycobacterial exposure or infection are less likely to occur with IGRAs than with tuberculin skin testing.

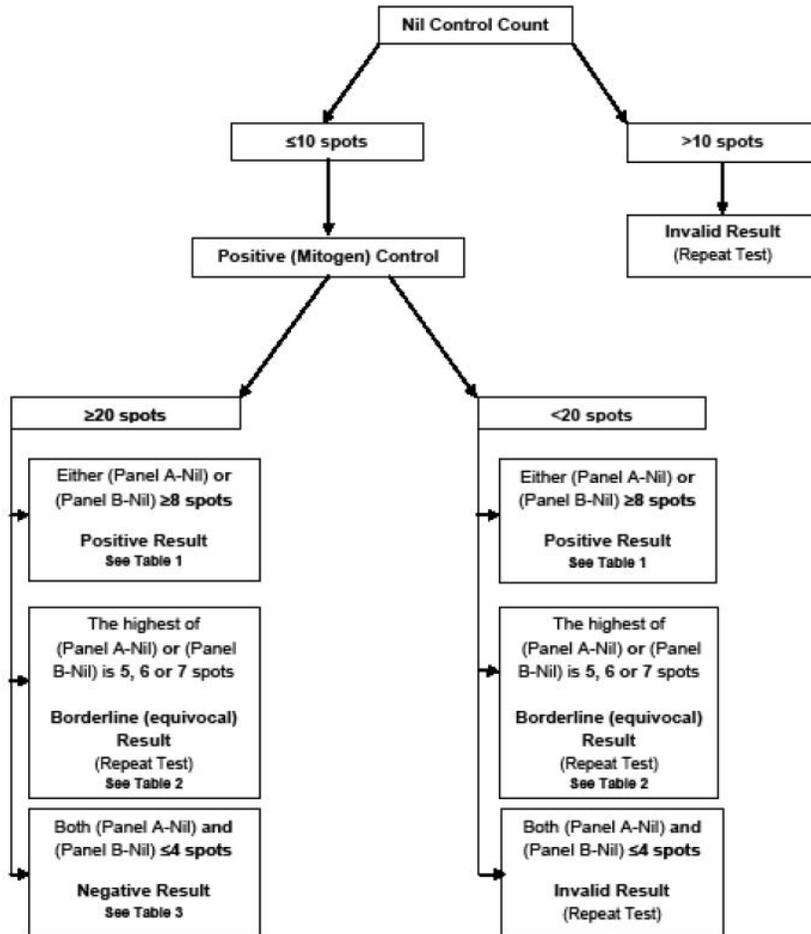
Despite these potential advantages, the IGRAs are imperfect tests for TB diagnosis. There is a large body of data demonstrating the association between a positive tuberculin skin test and the risk to develop active tuberculosis, but the data demonstrating a similar association between a positive IGRA and risk for active tuberculosis are much more limited. Additionally, every study comparing the two available IGRA tests has demonstrated a significant proportion of discordant results, the significance of which is unknown. This discordance means that the IGRAs are not interchangeable; switching among the IGRAs (or for that matter, between an IGRA and tuberculin skin testing) in the setting of serial testing may result in false "conversions" caused by discordance between the different tests used (as opposed to new TB infection). Furthermore, the cost of an IGRA is significantly greater than the cost of a tuberculin skin test.

Currently two IGRAs are approved for use in the United States by the Food and Drug Administration. The Quantiferon Gold in-tube® is an enzyme-linked immunosorbent assay-based test. The test measures the concentration of interferon gamma in whole blood in 3 separate tubes: a nil tube (negative control), a tube containing 3 TB antigens (ESAT-6, CFP-10, and TB7.7), and a tube containing phytohemagglutinin (a mitogen used as a positive control). Blood is drawn from the patient directly into each tube (about 1 ml of blood each), and the tubes must then be shaken vigorously. The tubes must be placed into an incubator at 37 C within 14 hours of the blood draw, and are then incubated for 16-24 hours. After incubation, a machine is used to measure the concentration of interferon gamma in each tube. The criteria for test interpretation are listed below (from the package insert, available at www.cellestis.com).

Nil [IU/mL]	TB Antigen minus Nil [IU/mL]	Mitogen minus Nil [IU/mL] ¹	QuantIFERON®-TB Gold IT Result	Report / Interpretation
≤ 8.0	≥ 0.35 and ≥ 25% of Nil value	Any	Positive²	<i>M. tuberculosis</i> infection likely
	< 0.35 OR ≥ 0.35 and < 25% of Nil value	≥ 0.5	Negative	<i>M. tuberculosis</i> infection NOT likely
> 8.0 ⁴	Any	< 0.5	Indeterminate³	Results are indeterminate for TB Antigen responsiveness
		Any		

The T-SPOT.TB® is an enzyme-linked immunospot test. The test measures the number of spots on a plate containing 4 different antigens: nil (negative control), 2 TB antigens (ESAT-6 and CFP-10, also called Panel A and Panel B), and phytohemagglutinin (positive control). Each spot theoretically represents a white blood cell that is secreting interferon gamma. Blood is drawn from the patient (8 mL for adults, 4 mL for children 2-9 years old, and 2 mL for children under 2 years) and then must be processed in the laboratory within 8 hours. The white blood cells are separated from the rest of the blood, and a standard number of white cells is placed into each plate. The cells are incubated with the antigens for 16-20 hours, and then further steps are used to develop the spots for each plate. The criteria for test interpretation are outlined in the diagram below (from the package insert at http://www.oxfordimmunotec.com/Technical_Documents_North_America).

Figure 3: Algorithm Flow Diagram



A brief comparison of the tuberculin skin test and the two IGRAs follows in the Table.

	Tuberculin skin test	Quantiferon Gold in-tube	T-SPOT.TB
Number of visits required	2*	1	1
Time frame to get blood to lab	N/A	<14 hours (can be incubated in portable incubator)	<8 hours
Need for additional processing of blood before incubation	N/A	No	Yes
Result format	mm of induration	Concentration of interferon gamma (IU/mL)	Number of spots on a plate
Reliability among observers	+/-	++	++
Test-retest variability	+	++	++
Potential as a “send out” test to distant labs	N/A	Yes	Yes (always done this way)
Cost to health department or healthcare facility	Reagent inexpensive, labor somewhat more	Moderately expensive	Moderately expensive
Cross-reacts with BCG, nontuberculous mycobacteria	Yes (mostly an issue in foreign-born populations)	No	No

* 4 visits may be necessary if 2-step testing is performed

Reporting of IGRA results

Reporting of IGRA results as “Positive,” “Negative,” or “Indeterminate” is clinically useful, but may be suboptimal in certain circumstances. Both clinically available IGRAs have a certain amount of test-retest variability (“wobble” — for example see Pai M et al., American Journal of Respiratory and Critical Care Medicine 2006 174: 349). Particularly when results are near the threshold for a positive test, repeat testing has a significant probability of producing a result on the other side of the threshold. This problem is of particular concern in the setting of repeat testing, when a patient may test negative at just below the threshold on one occasion, and then test positive at just above the threshold on another occasion solely due to inter-test variability.

To assist with test interpretation, the NC Tuberculosis Control Program recommends that laboratories report the following information:

Quantiferon Gold Plus®

- Nil tube interferon gamma concentration (IU/mL)
- TB1 antigen tube interferon gamma concentration (IU/mL)

- TB2 antigen tube interferon gamma concentration (IU/mL)
- TB1-Nil and TB2-nil values (difference, IU/mL)
- Criteria for a positive value (i.e. difference of ≥ 35 IU/mL and $>25\%$ of nil)
- Interpretation (“Positive,” “Negative,” “Indeterminate”)

T-SPOT.TB®

- Nil plate spots (number)
- TB antigen plate spots (number, both plates)
- Highest difference (TB antigen-nil)
- Criteria for a positive value (i.e. difference of ≥ 8 spots)
- Interpretation (“Positive,” “Negative,” “Indeterminate”)

Management of indeterminate results

Both IGRAs may yield indeterminate results, either due to high Nil background or an inadequate interferon gamma response to mitogen. Indeterminate results are more common in the setting of immunosuppression, but can also occur in apparently immunocompetent hosts. However, a significant proportion of persons with indeterminate results on one occasion will have a non-indeterminate result on repeat testing. Similarly, while an indeterminate result is associated with an anergic response to the tuberculin skin test, not all persons with an indeterminate response from an IGRA will have anergy in response to the tuberculin skin test.

Recommendations:

- If an indeterminate result is obtained from IGRA testing, repeating the same IGRA test should be considered.
- If an indeterminate result is obtained from one IGRA test (outside the setting of serial testing) performing a different IGRA test (if available) or a tuberculin skin test can be considered

Diagnosis of active tuberculosis

Like the tuberculin skin test, IGRAs are at best imperfect tools in the diagnosis of active TB. A recent meta-analysis estimated that the sensitivity of the tuberculin skin test among persons with active tuberculosis was 77%, the sensitivity of the Quantiferon Gold in-tube® was 70%, and the sensitivity of the T-SPOT.TB® was 90% (Pai M et al., *Annals of Internal Medicine* 2008; 149: 177). IGRAs will therefore be falsely negative in a significant proportion of persons with active TB. Also, IGRAs cannot discriminate between latent TB infection and active TB disease in a given patient. However, one study did demonstrate that IGRAs were useful in distinguishing children with TB cervical lymphadenitis from children with nontuberculous mycobacterial lymphadenitis (*Clin Infect Dis.* 2007; 45(3):322) in a low-incidence area.

Recommendations:

- Like the tuberculin skin test, IGRAs should not be relied upon to make or disprove the diagnosis of active TB in adults.
- IGRAs may be used in children as part of diagnostic algorithms for TB diagnosis, keeping in mind their imperfect test characteristics.
- Microbiologic diagnosis (culture) is the gold standard and should be aggressively pursued in both adults and children.

Appendix F

Quality Control Procedural Observation Checklist (from MMWR2 2005:54 (RR-17))

Appendix F. Quality control (QC) procedural observation checklists

Quality Control (QC) Procedural Observation Checklist for Placing Tuberculin Skin Tests (TSTs) — Mantoux Method		
Date _____	Trainer (QC by) _____	Trainee (TST placed by) _____
<div style="border: 1px solid black; padding: 2px; display: inline-block;"> Scoring: ✓ or Y = Yes X or N = No NA = Not Applicable </div>		
1. Preliminary		
<input type="checkbox"/> Uses appropriate hand hygiene methods before starting. <input type="checkbox"/> Screens patient for contraindications (severe adverse reactions to previous TST). [*] <input type="checkbox"/> Uses well-lit area.	<input type="checkbox"/> Holds needle bevel-up and tip at 5°–15° angle to skin. <input type="checkbox"/> Inserts needle in first layer of skin with tip visible beneath skin. <input type="checkbox"/> Advances needle until entire bevel is under the first layer of skin. <input type="checkbox"/> Releases stretched skin. <input type="checkbox"/> Injects entire dose slowly. <input type="checkbox"/> Forms wheal, as liquid is injected. <input type="checkbox"/> Removes needle without pressing area. <input type="checkbox"/> Activates safety feature of device per manufacturer's recommendations, if applicable.	<input type="checkbox"/> Places used needle and syringe immediately in puncture-resistant container without recapping needle. <input type="checkbox"/> Immediately measures wheal to ensure 6–10 mm in diameter (Actual wheal measurement _____mm). <input type="checkbox"/> If blood or fluid is present, blots site lightly with gauze or cotton ball. <input type="checkbox"/> Discards used gauze or cotton ball according to local standard precautions. <input type="checkbox"/> If the TST is administered incorrectly (too deeply or too shallow) and the wheal is inadequate (<6 mm), a new TST should be placed immediately. Applying the second TST on the other arm or in a different area of the same arm (at least 2 inches from the first site) is preferable so that the TST result will be easier to read. <input type="checkbox"/> Documents all information required by the setting (e.g., date and time of TST placement, person who placed TST, location of injection site and lot number of tuberculin). <input type="checkbox"/> Uses appropriate hand hygiene methods after placing TST.
2. Syringe[†] filled with exactly 0.1 mL of 5 tuberculin units (TU) purified protein derivative (PPD) antigen[§]		
<input type="checkbox"/> Removes antigen vial from refrigeration and confirms that it is 5 TU PPD antigen. [†] <input type="checkbox"/> Checks label and expiration date on vial. <input type="checkbox"/> Marks opening date on multidose vial. <input type="checkbox"/> Fills immediately after vial removed from refrigeration. <input type="checkbox"/> Cleans vial stopper with antiseptic swab. <input type="checkbox"/> Twists needle onto syringe to ensure tight fit. <input type="checkbox"/> Removes needle guard. <input type="checkbox"/> Inserts needle into the vial. <input type="checkbox"/> Draws slightly over 0.1 mL of 5 TU PPD into syringe. <input type="checkbox"/> Removes excess volume or air bubbles to exactly 0.1 mL of 5 TU PPD while needle remains in vial to avoid wasting of antigen. <input type="checkbox"/> Removes needle from vial. <input type="checkbox"/> Returns antigen vial to the refrigerator immediately after filling.	<input type="checkbox"/> Removes antigen vial from refrigeration and confirms that it is 5 TU PPD antigen. [†] <input type="checkbox"/> Checks label and expiration date on vial. <input type="checkbox"/> Marks opening date on multidose vial. <input type="checkbox"/> Fills immediately after vial removed from refrigeration. <input type="checkbox"/> Cleans vial stopper with antiseptic swab. <input type="checkbox"/> Twists needle onto syringe to ensure tight fit. <input type="checkbox"/> Removes needle guard. <input type="checkbox"/> Inserts needle into the vial. <input type="checkbox"/> Draws slightly over 0.1 mL of 5 TU PPD into syringe. <input type="checkbox"/> Removes excess volume or air bubbles to exactly 0.1 mL of 5 TU PPD while needle remains in vial to avoid wasting of antigen. <input type="checkbox"/> Removes needle from vial. <input type="checkbox"/> Returns antigen vial to the refrigerator immediately after filling.	<input type="checkbox"/> Places used needle and syringe immediately in puncture-resistant container without recapping needle. <input type="checkbox"/> Immediately measures wheal to ensure 6–10 mm in diameter (Actual wheal measurement _____mm). <input type="checkbox"/> If blood or fluid is present, blots site lightly with gauze or cotton ball. <input type="checkbox"/> Discards used gauze or cotton ball according to local standard precautions. <input type="checkbox"/> If the TST is administered incorrectly (too deeply or too shallow) and the wheal is inadequate (<6 mm), a new TST should be placed immediately. Applying the second TST on the other arm or in a different area of the same arm (at least 2 inches from the first site) is preferable so that the TST result will be easier to read. <input type="checkbox"/> Documents all information required by the setting (e.g., date and time of TST placement, person who placed TST, location of injection site and lot number of tuberculin). <input type="checkbox"/> Uses appropriate hand hygiene methods after placing TST.
3. TST administration site selected and cleaned		
<input type="checkbox"/> Selects upper third of forearm with palm up ≥2 inches from elbow, wrist, or other injection site. ^{**} <input type="checkbox"/> Selects site free from veins, lesions, heavy hair, bruises, scars, and muscle ridge. <input type="checkbox"/> Cleans arm site with antiseptic swab using circular motion from center to outside. <input type="checkbox"/> Allows site to dry thoroughly before administering antigen.	<input type="checkbox"/> Selects upper third of forearm with palm up ≥2 inches from elbow, wrist, or other injection site. ^{**} <input type="checkbox"/> Selects site free from veins, lesions, heavy hair, bruises, scars, and muscle ridge. <input type="checkbox"/> Cleans arm site with antiseptic swab using circular motion from center to outside. <input type="checkbox"/> Allows site to dry thoroughly before administering antigen.	<input type="checkbox"/> Selects upper third of forearm with palm up ≥2 inches from elbow, wrist, or other injection site. ^{**} <input type="checkbox"/> Selects site free from veins, lesions, heavy hair, bruises, scars, and muscle ridge. <input type="checkbox"/> Cleans arm site with antiseptic swab using circular motion from center to outside. <input type="checkbox"/> Allows site to dry thoroughly before administering antigen.
4. Needle inserted properly to administer antigen		
<input type="checkbox"/> Rests arm on firm, well-lit surface. <input type="checkbox"/> Stretches skin slightly. ^{††}	<input type="checkbox"/> Rests arm on firm, well-lit surface. <input type="checkbox"/> Stretches skin slightly. ^{††}	<input type="checkbox"/> Rests arm on firm, well-lit surface. <input type="checkbox"/> Stretches skin slightly. ^{††}
5. Explanation to the client regarding care instructions for the injection site		
<input type="checkbox"/> The wheal (bump) is normal and will remain about 10 minutes. <input type="checkbox"/> Do not touch wheal; avoid scratching. <input type="checkbox"/> Avoid pressure or bandage on injection site. <input type="checkbox"/> Rare local discomfort and irritation does not require treatment. <input type="checkbox"/> May wash with soap and water (without pressure) after 1 hour. <input type="checkbox"/> No lotions or liquids on site, except for light washing, as above. <input type="checkbox"/> Keeps appointment for reading.	<input type="checkbox"/> The wheal (bump) is normal and will remain about 10 minutes. <input type="checkbox"/> Do not touch wheal; avoid scratching. <input type="checkbox"/> Avoid pressure or bandage on injection site. <input type="checkbox"/> Rare local discomfort and irritation does not require treatment. <input type="checkbox"/> May wash with soap and water (without pressure) after 1 hour. <input type="checkbox"/> No lotions or liquids on site, except for light washing, as above. <input type="checkbox"/> Keeps appointment for reading.	<input type="checkbox"/> The wheal (bump) is normal and will remain about 10 minutes. <input type="checkbox"/> Do not touch wheal; avoid scratching. <input type="checkbox"/> Avoid pressure or bandage on injection site. <input type="checkbox"/> Rare local discomfort and irritation does not require treatment. <input type="checkbox"/> May wash with soap and water (without pressure) after 1 hour. <input type="checkbox"/> No lotions or liquids on site, except for light washing, as above. <input type="checkbox"/> Keeps appointment for reading.

^{*} Severe adverse reactions to the TST are rare but include ulceration, necrosis, vesiculation, or bullae at the test site, or anaphylactic shock, which is substantially rare. These reactions are the only contraindications to having a TST administered.

[†] Use a ¼–½-inch 27-gauge needle or finer, disposable tuberculin (preferably a safety-type) syringe.

[§] Prefilling syringes is not recommended. Tuberculin is absorbed in varying amounts by glass and plastics. To minimize reduction in potency, tuberculin should be administered as soon after the syringe has been filled as possible. Following these procedures will also help avoid contamination. Test doses should always be removed from the vial under strictly aseptic conditions, and the remaining solution should remain refrigerated (not frozen). Tuberculin should be stored in the dark as much as possible and exposure to strong light should be avoided. SOURCE: American Thoracic Society, CDC, Infectious Disease Society of America. Diagnostic standards and classification of tuberculosis in adults and children. Am J Respir Crit Care Med 2000;161:1376–95.

[†] Preventing tuberculin antigen and vaccine (e.g., Td toxoid) misadministration is important. Measures should include physical separation of refrigerated products, careful visual inspection and reading of labels, preparation of PPD for patient use only at time of testing, and improved record keeping of lot numbers of antigens, vaccines, and other injectable products. SOURCE: CDC. Inadvertent intradermal administration of tetanus toxoid-containing vaccines instead of tuberculosis skin tests. MMWR 2004;53:662–4.

^{**} If neither arm is available or acceptable for testing, the back of the shoulder is a good alternate TST administration site. SOURCE: National Tuberculosis Controllers Association, National Tuberculosis Nurse Consultant Coalition. Tuberculosis nursing: a comprehensive guide to patient care. Smyrna, GA: National Tuberculosis Controllers Association; 1997.

^{††} Stretch skin by placing nondominant hand of health-care worker (HCW) on patient's forearm below the needle insertion point and then applying traction in the opposite direction of the needle insertion. Be careful not to place the nondominant hand of the HCW opposite the administration needle if the patient is likely to move during the procedure, which might cause an accidental needle-stick injury. In children and others who are likely to move during the procedure, certain trainers prefer stretching the skin in the opposite direction of the needle insertion by placing the nondominant hand of the HCW under the patient's forearm. This method should not be used for persons with poor skin turgor.

Appendix F. (Continued) Quality control (QC) procedural observation checklists

Quality Control (QC) Procedural Observation Checklist for Reading Tuberculin Skin Test (TST) Results — Palpation Method

Date _____ Trainer (QC by) _____ Trainee (TST placed by) _____

Scoring: ✓ or Y = Yes X or N = No NA = Not Applicable

1. Preliminary

- _____ Uses appropriate hand hygiene methods before starting.
- _____ Keeps fingernails shorter than fingertips to avoid misreading TST result.
- _____ Keeps TST reading materials at hand (eyeliner pencil or ballpoint pen,* and ruler).
- _____ Uses well-lit area.
- _____ Inspects for the site of the injection.

2. Palpate — finding margin ridges (if any)

- _____ Palpates with arm bent at elbow at a 90° angle.
- _____ Lightly sweeps 2-inch diameter from injection site in four directions.
- _____ Uses zigzag featherlike touch.
- _____ Repeats palpation with arm bent at elbow at a 45° angle to determine presence or absence of induration.

If induration is present, continue with these steps†:

3. Placing marks

- _____ Holds palm over injection site.
- _____ Uses fingertips to find margins of the induration.
- _____ Marks the induration by placing small dots on both sides of the induration.
- _____ Inspects dots, repeats finger movements toward indurated margin, and adjusts dots if needed.
- _____ Marks dots transverse (perpendicular) to long axis of forearm.

4. Placing and reading ruler

- _____ Places the “0” ruler line inside the edge of the left dot. Reads the ruler line inside right dot edge (uses lower measurement if between two gradations on millimeter scale) (see Figure 1).
- _____ Uses appropriate hand hygiene methods after reading TST.

5. Documenting results

- _____ Records all TST results in millimeters, even those classified as negative. Does not record only as “positive” or “negative.” Records the absence of induration as “0 mm.”
- _____ Correctly records results in mm; only a single measured induration in mm should be recorded.
 Trainee’s measurement _____ mm.
 Trainer’s (gold standard) measurement _____ mm.
 Trainee’s result within 2 mm of gold standard reading?§
 Yes _____ No _____

NOTE: In rare instances, the reaction might be severe (vesiculation, ulceration, or necrosis of the skin). Report severe adverse events to the FDA MedWatch Adverse Events Reporting System (AERS), telephone: 800-FDA-1088; fax: 800-FDA-0178; <http://www.fda.gov/medwatch> report form 3500, Physicians’ Desk Reference.

* A fine-tipped eyeliner pencil or ballpoint pen can be used as a marker. An eyeliner pencil is useful for TST training and for blinded independent duplicate readings (BIDRs) because the dots are easy to remove with a dot of lubricant (e.g., baby oil). Alternative TST result reading methods have been described, including the pen method.

† If induration is not present, record the TST result as 0 mm and go to the end of this form (Documenting results).

§ For example, if the TST trainer reads the TST result (the gold standard reading) as 11 mm, the trainee’s TST reading should be between 9–13 mm to be considered correct. Only a single measured induration in millimeters should be recorded.

Sample Standing Order: Purified Protein Derivative (PPD) Placement

Assessment: This standing order shall be initiated to place a PPD if the PHN has completed a TB program orientation which includes training on the administration and reading of PPD's in any of the following situations and if the following subjective and objective findings exist:

- when an individual is a contact to active tuberculosis (TB)
- in individuals with signs and or symptoms of active tuberculosis (abnormal chest x-ray, positive AFB smear or culture, productive cough, fever, night sweats, shortness of breath, chest pain, unexplained appetite loss, unexplained weight loss, or unexplained fatigue), or
- Individual has a new positive HIV test

Subjective findings:

Screening for Mycobacterium tuberculosis is:

- Requested by patient, or
- Requested by medical provider, or
- Required by communicable disease/TB rules

Objective findings:

. Medical record indicates:

- The patient has not received a live virus vaccine in the last 30 days.
- The patient has no documentation of a positive Interferon Gamma Release Assay (IGRA) or PPD.

Implementation:

Administering a PPD

1. Tuberculin skin test (TST) shall be performed using 0.1 cc of 5TU Purified Protein Derivative (PPD).
2. Use tuberculin syringe with 3/8 inch, 26-27 gauge needle
3. Clean volar or flexor surface of left forearm approximately 2-4 inches below the elbow: allow to dry completely
4. Give intradermal injection with needle bevel up. A tense white wheal 6-10 mm in diameter should be produced when the TST is accurately administered.
5. Repeat injection at another site at least 2 inches from original site if antigen is lost or injection is given too deeply. Specify location of the retest in the record.
6. If the use of the left arm is not feasible due to injury, deformity, etc, the PPD may be administered in the volar or flexor region of the right forearm and a notation of the site change recorded on the client's chart.
7. Administer TST prior to or simultaneously with live virus vaccines. Client may not receive another TST for 4-6 weeks after live virus vaccines

Reading a PPD

1. Read TST 48 – 72 hours after placement, preferably at 72 hours
 - a. Instruct patient to return to the health department if induration occurs after the TST is read
 - b. Positive TST reactions occurring after 72 hours are considered valid.

- c. Negative TST reactions should be repeated when individuals fail to return to return within 72 hours.
2. Locate induration (not redness) by palpating in a crosswise motion. Measure transversely to the long axis of the forearm and record this as a single measurement.
3. Record reaction in mm (example: 0 mm, 16 mm) and document date of reading and signature of the person reading the test.

Interpretation of PPD results: A skin test is considered positive based on the risk factors of the patient.

1. A reaction of ≥ 5 mm induration is considered positive for:
 - a. Close contacts to an individual with known or suspected infectious tuberculosis within the past 2 years.
 - b. Those suspected of having active TB disease based on clinical and/or chest x-ray evidence.
 - c. Individuals with HIV infection.
 - d. Individuals with fibrotic changes on chest x-ray consistent with prior TB.
 - e. Individuals with organ transplants and other immunosuppressed patients, including those receiving ≥ 15 mg per day of Prednisone for one month or longer or persons taking or considering taking tumor necrosis factor (TNF) inhibitors such as etanercept (Enbrel®), infliximab (Remicade®), adalimumab (Humira®) or anakinra (Kineret™), or other similar biologic immunosuppressive agents.
2. A reaction of ≥ 10 mm induration is considered positive for:
 - a. Children younger than 4 years of age
 - b. Foreign-born individuals from high-prevalence countries, e.g. Asia, Africa, Caribbean, Latin America, Mexico, South America, Pacific Islands or Eastern Europe. Low-prevalence countries for TB disease are USA, Canada, Japan, Australia, Western Europe and New Zealand
 - c. HIV-negative individuals who inject illicit drugs or use crack cocaine
 - d. Individuals with medical conditions that have been reported to increase the risk of tuberculosis disease once infected:
 - i. diabetes mellitus
 - ii. chronic malabsorption syndrome
 - iii. chronic renal failure
 - iv. leukemia, lymphomas, Hodgkin's disease
 - v. cancer of the head or neck
 - vi. weight loss of $> 10\%$ below ideal body weight
 - vii. silicosis
 - viii. gastrectomy, or jejunioileal bypass
 - e. Residents and staff in long-term care facilities.
 - f. Health care workers
 - g. Inmates in the Department of Corrections
 - h. Staff with direct inmate contact in the Department of Corrections and jails
 - i. Employees of HIV/AIDS adult daycare centers
 - j. Homeless shelter residents, employees and volunteers
 - k. Individuals who increase their mm reading by 10mm or more within 2 years (converter)

- l. Mycobacteriology lab personnel
 - m. Children and adolescents exposed to high-risk adults (homeless, substance abuse, incarcerated, HIV positive)
 - n. Persons who have traveled outside the US and stayed with family and friends who live in high incidence areas, for greater than 1 month cumulatively
3. A reaction of ≥ 15 mm induration is considered positive for:
- a. Individuals who do not have any of the above risk factors.

Nursing Action:

1. Store the tuberculin skin test solution in the vaccine refrigerator. Tuberculin solution can be adversely affected by exposure to light or temperature extremes
2. Advise the patient to return to the health department between 48 and 72 hours to have the PPD read.
3. Instruct the patient not to scratch the PPD site or place a Band-Aid over the site of injection.

Criteria for calling physician:

1. Contact physician if there is any question about whether to carry out any part of this standing order.
2. Contact physician if the individual has an allergic reaction to the PPD.

Follow-up requirements:

If the PPD result is positive:

1. Obtain a posterior/anterior view chest x-ray for individuals 5 years or older and add a lateral view chest x-ray for children less than 5 for all individuals with an induration considered positive based on the above criteria. Appropriate shielding should be used for all pregnant women,
2. Complete a TB Epidemiological form (DHHS 1039).
3. Obtain a HIV test unless the individual refuses.
4. Notify the health department TB nurse about all patients determined to have a positive PPD for further follow-up. (This may be modified if the local health department has a different procedure for positive PPD follow-up.

Resources:

- North Carolina TB Control Manual 2012 edition.

Legal Authority:

- Nurse Practice Act, G.S. 90-171.20 (7) (f) & (8) (c)

Date written: _____

Approved by: _____

Date: _____

Approved by: _____

Date: _____

Approved by: _____

Date: _____

Sample Standing Order: Obtaining an Interferon Gamma Release Assay (IGRA)

Assessment:

This standing order shall be initiated by a PHN who has completed a TB program orientation. This standing order will be used to obtain an IGRA in any of the following situations and if subjective and objective findings exist:

- when an individual is a contact to active tuberculosis (TB), or
- in individuals with signs and or symptoms of active tuberculosis (abnormal chest x-ray, positive AFB smear or culture, productive cough, fever, night sweats, shortness of breath, chest pain, unexplained appetite loss, unexplained weight loss, or unexplained fatigue), or
- Individual has a new positive HIV test

Subjective findings:

Screening for Mycobacterium tuberculosis is:

- Requested by patient, or
- Requested by medical provider, or
- Required by communicable disease/TB rules

Objective findings:

Medical record indicates:

- The patient has not received a live virus vaccine in the last 30 days.
- The patient has no documentation of a positive Interferon Gamma Release Assay (IGRA).

Implementation:

Refer to or insert the lab test procedure for drawing the blood/shipping to reference lab, etc.

Nursing Action:

- Review the IGRA lab result.
 - If positive, give results to the patient and proceed with the follow-up requirements below.
 - If results are negative, provide results to the patient.
 - If results are indeterminate, or inconclusive, repeat the IGRA as soon as possible.

Criteria for calling physician:

- Call the physician if there is any question about whether to implement the standing order.
- Call the physician for further guidance if an IGRA is repeated because the results are indeterminate and the second result is also indeterminate.

Follow-up requirements:

If the IGRA result is positive:

5. Obtain a posterior/anterior view chest x-ray for individuals 5 years or older and add a lateral view chest x-ray for children less than 5. Provide appropriate shielding for pregnant women.
6. Complete a TB Epidemiological form (DHHS 1039).

7. Obtain a HIV test unless the individual refuses.
8. Notify the health department TB nurse about all patients determined to have a positive IGRA for further follow-up. (This may be modified if the local health department has a different procedure for positive IGRA follow-up.)

Resources:

- North Carolina TB Control Manual 2018 edition.

Legal Authority:

- Nurse Practice Act, G.S. 90-171.20 (7) (f) & (8) (c)

Date written: _____

Approved by: _____

Date: _____

Approved by: _____

Date: _____

Approved by: _____

Date: _____